DATA EVALUATION RECORD

DIAMINOTRIAZINE (BCS-AA10717)

Study Type: OPPTS 870.3700b [§83-3b]; Developmental Toxicity Study in Rabbits

Work Assignment No. 5-01-203 F (MRID 47443292)

Prepared for
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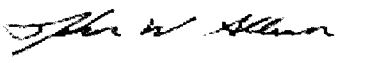
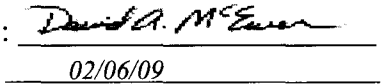
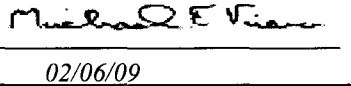
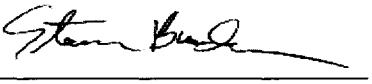
Primary Reviewer: John W. Allran, M.S.	Signature:  Date: *02/06/09*
Secondary Reviewer: David A. McEwen, B.S.	Signature:  Date: *02/06/09*
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature:  Date: *02/06/09*
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature:  Date: *02/06/09*

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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study in Rabbits (gavage); OPPTS 870.3700b [' 83-3b]; OECD 414.

PC CODE: 129108 **DP BARCODE:** D356856
TXR#: 0054980

TEST MATERIAL (PURITY): Diaminotriazine (93.14% a.i.)

SYNONYMS: BCS-AA10717; AE 1170437; N-[(1 R,2S)-2,6-dimethyl-2,3-dihydro-1 H-inden-1 -yl]-6-[(1 R)-1 -fluoroethyl]-1,3,5-triazine-2,4-diamine

CITATION: Wason, S. (2008) BCS-AA10717: Developmental toxicity study in the rabbit by gavage. Bayer CorpScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 07017, January 31, 2008. MRID 47443292. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Straße 50, Monheim, Germany

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 47443292), Diaminotriazine (93.14%; Batch # EFIM000511) in 0.5% aqueous methylcellulose 400 was administered via daily oral gavage in a dose volume of 4 mL/kg to 23 naturally-mated presumed pregnant New Zealand White rabbits/dose group at doses of 0, 10, 25, or 60 mg/kg/day from gestation days (GD) 6-28. On GD 29, all surviving maternal rabbits were euthanized; the uterus of each rabbit was removed via cesarean section and its contents examined. The fetuses were examined for external, visceral, and skeletal malformations and variations.

At 60 mg/kg/day, one female (RR4F0461) was killed for humane reasons on GD 27 after having aborted. This animal had decreased food consumption and lost 0.17 kg between GD 24-26. Clinical signs consisted of few feces on GD 26 and 27. Pale liver was noted at necropsy. There were no other treatment-related mortalities.

At 60 mg/kg/day, 3/23 dams had mucoid feces on two or more occasions from GD 16 onwards, compared to 0 controls. Additionally at this dose, 12/23 females had few feces on one or more occasions compared to 2/23 controls. Mean maternal body weight gain at 60 mg/kg/day was significantly ($p<=0.05$) decreased by 32% for the overall (GD 6-29) treatment period, compared to controls. Corrected (for gravid uterine weight) body weight gain was significantly ($p<=0.01$) decreased by 200%. Corresponding to the decreased body weight gains at this dose, food consumption was decreased by 10-30% at each of the intervals throughout treatment, compared

to controls. These decreases were significantly different ($p<=0.05$) from controls for GD 6-8, 8-10, 22-26, and 26-29. Although liver weights in the treated groups were comparable to controls, pale liver was noted in 3/23 females, and white foci on the liver were found in 2/23 dams at 60 mg/kg/day, compared to 0 controls.

There were no effects on treatment on the 10 or 25 mg/kg/day dams.

The maternal LOAEL is 60 mg/kg/day based on abortion in a single dam, decreased body weight gains, decreased food consumption, and macroscopic findings in the liver (pale liver, white foci). The maternal NOAEL is 25 mg/kg/day.

There were no premature deliveries or complete litter resorptions. There were no effects of treatment on the numbers of litters, live fetuses, dead fetuses, early resorptions, or late resorptions. Additionally, sex ratio and post-implantation losses in the treated groups were comparable to controls.

There were no treatment-related effects on fetal growth or development as evidenced by fetal body weights in the treated groups that were comparable to controls and the absence of any effects on ossification of the skeleton.

There were no treatment-related external, visceral, or skeletal malformations or variations.

The developmental LOAEL was not observed. The developmental NOAEL is 60 mg/kg/day.

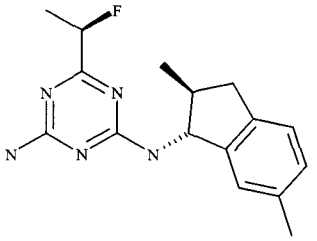
This study is classified **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3700b; OECD 414) for a developmental toxicity study in rabbits.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Diaminotriazine (BCS-AA10717)

Description:	Light beige powder
Batch #:	EFIM000511
Purity:	93.14% a.i.
Compound stability:	Stable in the vehicle for up to 30 days refrigerated
CAS #:	730979-19-8
Structure:	

2. **Vehicle:** 0.5% aqueous methylcellulose 400

3. **Test animals**

Species:	Rabbit
Strain:	New Zealand White Crl:KBL (NZW)
Age/ weight range at GD 0:	Approximately 18 weeks old; 3.15-3.85 kg females
Source:	Charles River Laboratories (Chatillon-sur-Chalaronne, France)
Housing:	Individually in suspended, stainless steel, wire mesh cages
Diet:	110 C-10 pelleted animal diet from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France), *ad libitum*. Additionally, a small amount of kale was placed in the bottom of each cage once or twice per week.
Water:	Filtered and softened tap water, *ad libitum*
Environmental conditions:	**Temperature:** 17-21°C **Humidity:** 40-70% **Air changes:** 10-15/hour **Photoperiod:** 16 hours light : 8 hours dark
Acclimation period:	4 or 5 days

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: May 26, 2007 End: July 5, 2007

2. **Mating:** Females were mated with stock males of the same strain and supplier. The day of insemination was designated as gestation day (GD) 0.

3. **Animal assignment:** On each day of mating, the females were randomly assigned, stratified by body weight, to the dose groups shown in Table 1. Body weights were checked after randomization to ensure similar means among all groups.

TABLE 1. Animal assignment [a]

Dose (mg/kg/day)	0	10	25	60
No. females	23	23	23	23

a Data were obtained from page 16 of MRID 47443292.

4. **Dose-selection rationale:** The doses were selected based on results of a range-finding study (SA 04121)[1], in which pregnant rabbits were administered the test formulations at doses of 0, 10, 25, 60, or 120 mg/kg/day from GD 6 to 28. Due to overt maternal toxicity, the 120 mg/kg/day group was terminated early. At 60 mg/kg/day, there was a maternal body weight loss of 0.05 kg between GD 6-8 and 0.02 kg between GD 8-10. Maternal food consumption was decreased by 13-35% at this dose for each interval between GD 6 and GD 18 compared to controls.

5. **Dose preparation and analysis:** The test formulations were prepared six times during the study by suspending the appropriate amount of the test substance in aqueous 0.5% (w/v) methylcellulose 400. The dosing suspensions were stored at approximately 5 ± 3°C until use. Homogeneity (top, middle, and bottom) of the test material in the vehicle was verified at concentrations of 2.5 and 15 g/L (equivalent to the low and high doses of 10 and 60 mg/kg/day, respectively) from the first formulations prepared for the study. Stability of the test material in the vehicle at concentrations of 0.127 and 250 g/L was determined in a previous study (SA 04129)[2], and the test formulations were found to be stable for 30 days under similar conditions to those of the current study. Concentration analyses were performed on the suspensions for each dose level from all preparations used during the study; the average values from the homogeneity checks were used as the measured concentrations for the low and high dose from the first preparation.

Results

Homogeneity: 92-102% nominal; 3.4-4.9% CV

Stability: 98-107% nominal after 30 days refrigerated (5 ± 3°C)

Concentration: 92-101% nominal

The analytical data indicate that the test substance was homogeneous and stable in the suspensions and that the variation between the nominal and actual dosages to the animals was acceptable.

6. **Dose administration:** Doses were administered daily by oral gavage at a volume of 4 mL/kg body weight from GD 6 to 28 and were based on the animal's most recent body weight. Control animals received an equivalent volume of vehicle alone. The suspensions were mixed continuously with an electromagnetic stirrer before and during dosing.

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1 McElligott A. (2005): AE 1170437, Range finding study for developmental toxicity in the rabbit by gavage, Bayer CropScience, Sophia Antipolis, France (SA 04121).

2 Clamagirand V. (2004): AE 1170437, Stability in aqueous methylcellulose, Bayer CropScience, Sophia Antipolis, France (SA 04129).

C. OBSERVATIONS

1. **Maternal observations and evaluations:** Cage-side checks for dead or moribund animals were performed twice daily (morning and afternoon) during the week and once daily on weekends and public holidays. Clinical signs were recorded daily from GD 2-29. Body weights were recorded on GD 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 29. Mean food consumption (g/rabbit/day) was reported for GD 3-6, 6-8, 8-10, 10-14, 14-18, 18-22, 22-26, and 26-29.

   On GD 29, all surviving females were euthanized by intravenous injection of Dolethal® (18.22 g/100 mL, sodium pentobarbital) and subjected to a gross necropsy. The liver from each dam was weighed. A portion of the liver from each dam was retained in 10% neutral buffered formalin for possible histological examination; however, the retained liver samples were discarded without examination. The reproductive tract was excised, and the gravid uterus was weighed. The numbers of corpora lutea, implantations, early resorptions, late resorptions, live fetuses, and dead fetuses were recorded. Resorptions were classified as early or late according to the criteria of Gleich and Frohberg (1977)[3]. An early resorption is defined as one for which it is not possible to macroscopically distinguish placental remnants from fetal remnants; whereas for late resorptions, a distinct discrimination between placental and fetal remnants is possible. Dead fetuses were defined as a dead conceptus with distinct digits on the fore and hindpaws. Runt fetuses were defined as live fetuses weighing less than 28 grams at the time of the cesarean section. Uterine horn(s) without visible implantations were immersed in a 2% solution of sodium hydroxide for approximately 2 hours, followed by immersion in 10% buffered formalin for a further 2 hours according to the methods of Sumida (1964)[4], in order to visualize any sites which were not apparent.

2. **Fetal evaluations:** Each live fetus was killed by subcutaneous injection (0.01 ml/fetus) of Dolethal® (18.22 g/100 mL, sodium pentobarbital), weighed, and subjected to an external examination. When possible, the body weights of dead fetuses were also recorded. After internal examination of the neck, the heads of approximately half of the live fetuses from each litter were immersed in Bouin's fluid for subsequent internal examination. The bodies of all of the fetuses were sexed and dissected for visceral abnormalities. Fetuses were then fixed in absolute ethanol, stained using a modification of the Staples and Schnell staining technique, and subjected to skeletal examination. Structural deviations were classified as either malformations or variations. A malformation is defined as a permanent structural change that is likely to adversely affect the survival or health; whereas a variation is a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health (this might include a delay in growth or morphogenesis that has otherwise followed a normal pattern of development).

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3 Gleich J. and Frohberg H. (1977): General teratological techniques. 91-102 in "Methods in prenatal toxicology: evaluation of embryotoxic effects in experimental animals", Teratology workshop, Berlin, April 1977, Neubert D., Merker H.J., Krvasigroch T.E., Georg Miene Publishers, Stuttgart.

4 Sumida H. et. al. (1994): Study on the implantation sites in rabbits, *Exp. Anim.* 43 (4), 585-588.

D. DATA ANALYSIS

1. **Statistical analyses:** Data from non-pregnant animals were not included in group mean calculations of any maternal parameters. The following statistical analyses were performed:

Parameters	Statistical tests
Body weight gains Corrected body weight gain Numbers of: corpora lutea implantations early resorptions late resorptions	Bartlett's test for homogeneity of variances. If variances were homogeneous, analysis of variance (ANOVA) was performed. If ANOVA revealed significant differences among groups, Dunnett's test (2-sided) was conducted for pair-wise comparisons of the treated groups with controls. If variances were not homogeneous, Kruskal-Wallis test was performed. If Kruskal-Wallis revealed significant differences among groups, Dunn test (2-sided) was conducted for pair-wise comparisons of the treated groups with controls.
Food consumption Liver weight	Data were analyzed in the same manner as body weight gain data above, except that, if Bartlett's test indicted that variances were not homogeneous, a log transformation was attempted to achieve homogeneity. If homogeneity was achieved, the analyses were performed on log-transformed data.
Pre-implantation loss Post-implantation loss	Data were analyzed in the same manner as body weight gain data above, except that, if Bartlett's test indicted that variances were not homogeneous, an arcsine transformation was attempted to achieve homogeneity. If homogeneity was achieved, the analyses were performed on the transformed data.
Number of live fetuses Number of dead fetuses	Fisher's exact test
Fetal sex ratio	Chi-square test
Fetal body weight	Bartlett's test for homogeneity of variances. If not homogeneous, a log transformation was applied to attempt to achieve homogeneity. If variances were homogeneous, differences among group means were tested using a Mixed Linear Model (Proc Mixed in SAS), with treatment as the fixed effect and the dam as the random effect (in order to account for the fact that fetuses within litters are not statistically independent). If the F test revealed significant differences among groups, Dunnett's test (2-sided) was conducted for pair-wise comparisons of the treated groups with controls. If variances were not homogeneous even after log transformation, Kruskal-Wallis test was performed. If Kruskal-Wallis revealed significant differences among groups, Dunn test (2-sided) was conducted for pair-wise comparisons of the treated groups with controls.

Significance was denoted at $p \leq 0.05$ and $p \leq 0.01$. The statistical methods were considered appropriate.

2. **Indices:** The following indices were calculated on a per litter basis:

Pre-implantation loss (%) = (# corpora lutea – # implantations)/ # corpora lutea x 100

Post-implantation loss (%) = (# implantations – # live fetuses)/ # implantations x 100

3. **Historical control data:** Historical control data from cesarean section and fetal examinations were provided from 10 rabbit studies conducted in-house between March 2000 and September 2006 (SA 00049, SA 02046, SA 02056, SA 03131, SA 03349, SA 03350, SA 04196, SA 04279, SA 05014, and SA 05042).

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality:** At 60 mg/kg/day, one female (RR4F0461) was killed for humane reasons on GD 27 after having aborted. This animal had decreased food consumption and lost 0.17 kg between GD 24-26. Clinical signs consisted of few feces on GD 26 and 27. Pale liver was noted at necropsy.

   There were no deaths at 25 mg/kg/day.

   At 10 mg/kg/day, one female (RR2F0414) was killed for humane reasons on GD 22. This female had eaten very little food and lost 0.37 kg in body weight between GD 12-22. Few/no feces were observed between GD 16-22. No treatment-related findings were noted at necropsy. Because there were no treatment-related findings at 25 mg/kg/day, this death was considered to be incidental. Another dam at this dose (RR2F0414) was euthanized on GD 12 following a gavage error (liquid was found in the trachea at necropsy).

   One control female (RR1F0408) was euthanized on GD 21 following what was assumed to be a gavage error. There were no clinical signs in this animal prior to death; at necropsy, the lungs were mottled with multiple foci.

2. **Clinical signs:** At 60 mg/kg/day, 3/23 dams had mucoid feces on two or more occasions from GD 16 onwards, compared to 0 controls (Table 2). Additionally at this dose, 12/23 females had few feces on one or more occasions compared to 2/23 controls. Although the incidences of few feces at 10 and 25 mg/kg/day were increased over controls, these observations were considered incidental because they were not dose-dependent. There were no other treatment-related clinical observations.

TABLE 2. Selected clinical signs (# affected) [a]

Observation	Dose in mg/kg bw/day (# of Dams)			
	0 (23)	10 (22)	25 (23)	60 (23)
Feces, mucoid	0	0	0	3
Feces, few	2	9	5	12

a Data were obtained from Table 2 on page 44 of MRID 47443292.

3. **Body weight gains:** At 60 mg/kg/day, mean maternal body weight gain was significantly ($p \leq 0.05$) decreased by 32% for the overall (GD 6-29) treatment period, compared to controls (Table 3). Corrected (for gravid uterine weight) body weight gain was significantly ($p \leq 0.01$) decreased by 200%. Body weight gains and corrected body weight gains at 10 and 25 mg/kg/day were comparable to controls.

TABLE 3. Mean (∀SD) maternal body weight gains (kg) [a]

Parameter/Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (19-20)	10 (17-19)	25 (22-23)	60 (21-22)
Pretreatment: GD 3-6	0.04 ± 0.068	0.01 ± 0.067	0.02 ± 0.072	0.04 ± 0.071
Treatment: GD 6-29	0.44 ± 0.121	0.39 ± 0.109	0.40 ± 0.130	0.30 ± 0.207* (↓32)
Corrected BW gain: GD 6-29 [b]	-0.08 ± 0.145	-0.11 ± 0.164	-0.15 ± 0.129	-0.24 ± 0.188** (↓200)

a Data were obtained from Table 3 on pages 49-51 of MRID 47443292. Percent differences from the control group, calculated by the reviewers, are included in parentheses.
b Corrected body weight gain = body weight on GD 29 – body weight on GD 6 – gravid uterine weight
* Significantly different from the control at $p \leq 0.05$
** Significantly different from the control at $p \leq 0.01$

4. **Food consumption:** At 60 mg/kg/day, maternal food consumption was decreased by 10-30% at each of the intervals throughout treatment, compared to controls (Table 4). These decreases were significantly different ($p \leq 0.05$) from controls for GD 6-8, 8-10, 22-26, and 26-29. Food consumption at 10 and 25 mg/kg/day was comparable to controls.

TABLE 4. Mean (∀SD) maternal food consumption (g/animal/day) [a]

Parameter/Interval		Dose in mg/kg bw/day (# of Dams)			
		0 (15-20)	10 (14-19)	25 (19-23)	60 (19-22)
Pretreatment: GD 3-6		166.9 ± 30.77	161.0 ± 27.30	160.4 ± 40.44	166.0 ± 34.77
Treatment:	GD 6-8	175.1 ± 42.23	171.3 ± 24.73	173.8 ± 32.28	139.3 ± 49.28* (↓20)
	GD 8-10	184.6 ± 19.67	176.8 ± 19.92	180.7 ± 34.28	148.0 ± 49.66* (↓20)
	GD 10-14	181.7 ± 22.85	168.5 ± 39.12	169.7 ± 47.90	154.2 ± 48.30 (↓15)
	GD 14-18	195.7 ± 29.89	170.5 ± 56.42	174.6 ± 30.03	161.8 ± 50.06 (↓17)
	GD 18-22	194.5 ± 33.72	174.4 ± 52.40	184.6 ± 31.25	175.0 ± 29.86 (↓10)
	GD 22-26	152.1 ± 38.23	138.5 ± 35.86	130.3 ± 30.56	115.4 ± 32.98** (↓24)
	GD 26-29	126.5 ± 33.16	114.9 ± 33.88	104.8 ± 27.25	88.8 ± 47.95** (↓30)

a Data were obtained from Table 4 on pages 53-54 of MRID 47443292. Percent differences from the control group, calculated by the reviewers, are included in parentheses.
* Significantly different from the control at $p \leq 0.05$
** Significantly different from the control at $p \leq 0.01$

5. **Liver weights:** The liver weights in the treated groups were comparable to controls.

6. **Gross pathology:** At 60 mg/kg/day, pale liver was noted in 3/23 females, and white foci on the liver was found in 2/23 rabbits, compared to 0 controls. There were no other macroscopic findings that could be attributed to treatment.

7. **Cesarean section data:** Summary data from the cesarean sections are presented in Table 5. There were no premature deliveries or complete litter resorptions. There were no effects of treatment on the numbers of litters, live fetuses, dead fetuses, early resorptions, or late resorptions. The number of dead fetuses was increased at 10 mg/kg/day (14 fetuses) compared to controls (6 fetuses); however, this finding was unrelated to dose. Additionally, fetal body weights, sex ratio, and post-implantation losses in the treated groups were comparable to controls.

TABLE 5. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	10	25	60
# Animals assigned (mated)	23	23	23	23
# Animals pregnant	20	19	23	22
Pregnancy rate (%)	87	83	100	96
# Nonpregnant	3	4	0	1
Maternal wastage				
No. found dead	0	0	0	0
No. euthanized *in extremis*	1	2	0	0
No. died pregnant	1	2	0	0
No. died nonpregnant	0	0	0	0
No. aborted	0	0	0	1
No. premature delivery	0	0	0	0
Total no. corpora lutea [b]	206	190	266	237
Corpora lutea/dam	10.8 ± 2.0	11.2 ± 2.8	11.6 ± 2.4	11.3 ± 2.0
Total no. implantations [b]	182	162	234	210
(Implantations/dam)	9.6 ± 2.4	9.5 ± 2.9	10.2 ± 1.9	10.0 ± 1.6
Total no. litters	19	17	23	21
Total no. live fetuses	162	137	213	195
(Live fetuses/dam)	8.5 ± 2.3	8.1 ± 2.6	9.3 ± 1.5	9.3 ± 1.5
Total no. dead fetuses	6	14*	11	12
(Dead fetuses/dam)	NP	NP	NP	NP
Total no. resorptions [b]	14	11	10	3
Early [b]	12	0	6	3
Late [b]	2	1	4	0
Resorptions/dam				
Early	0.6 ± 1.3	0.6 ± 0.8	0.3 ± 0.5	0.1 ± 0.4
Late	0.1 ± 0.3	0.1 ± 0.2	0.2 ± 0.4	0.0 ± 0.0
Complete litter resorptions	0	0	0	0
Mean fetal weight (g), sexes combined	40.8 ± 6.0	41.0 ± 6.5	39.7 ± 5.8	38.6 ± 5.8
males	41.4 ± 6.2	41.4 ± 6.9	40.4 ± 5.9	38.6 ± 6.2
females	40.3 ± 5.8	40.6 ± 6.0	38.9 ± 5.5	38.7 ± 5.3
Sex ratio (% male)	46.6 ± 13.0	49.3 ± 17.7	56.0 ± 16.7	54.9 ± 14.7
Pre-implantation loss (%)	12.0 ± 13.5	15.3 ± 15.1	11.0 ± 10.5	10.3 ± 12.2
Post-implantation loss (%)	10.1 ± 12.8	15.2 ± 12.2	8.2 ± 8.4	6.7 ± 8.7

a Data were obtained from Table 1 on page 42, Table 7 on pages 60-62, and Appendix E on pages 129-132 of MRID 47447813.

b Tabulated by the reviewers from data presented in Appendix F on pages 105-108 of the study report.

NP Not provided

* Significantly different from the control at $p \leq 0.05$

B. DEVELOPMENTAL TOXICITY

1. **External examinations:** All external findings are presented in Table 6. There were no treatment-related external malformations or variations. Malformations were only observed in one fetus at 10 mg/kg/day and three fetuses at 25 mg/kg/day. No malformations were noted at 60 mg/kg/day. Fetuses that were considered runts (weighing less than 28 g) were found in all dose groups, including the controls, at a low incidence and in a manner unrelated to dose. At 10 mg/kg/day, one hindpaw of one fetus was malrotated outward, with edema and a hematoma noted; it was suspected that this hindpaw was broken. No other external malformations or variations were observed.

TABLE 6. External findings [% fetuses (litters) affected] [a]

Observation	Dose (mg/kg bw/day)			
	0	10	25	60
No. fetuses (litters) examined	162 (19)	137 (17)	213 (23)	195 (21)
No. heads (litters) examined	76 (19)	65 (17)	99 (23)	92 (21)
Malformations				
Skull, neck and wall, thoracic edema	---	---	0.5 (4.3)	---
Abdomen, swollen	---	0.6 (5.9) [b]	---	---
Thoracic cavity, short	---	0.6 (5.9) [b]	---	---
Spina bifida (aperta)	---	---	0.3 (4.3)	---
Omphalocele	---	---	0.4 (4.3)	---
Variations				
Runt (body weight ≤28 g)	2.0 (21.1)	3.5 (23.5)	2.1 (21.7)	4.1 (19.0)
Hindpaw, malrotated outward	---	0.6 (5.9) [c]	---	---
edema and hematoma (unilateral)	---	0.6 (5.9) [c]	---	---
Skull, hematoma	---	0.7 (5.9)	---	---

a Data were obtained from Table 9 on page 69 of MRID 47443292.
b Observed in the same fetus
c Observed in the same fetus; it was stated that it was suspected that this hindpaw was broken.
--- No animals affected (i.e., zero incidence)

2. **Visceral examinations:** All visceral malformations and selected visceral variations are presented in Table 7. There were no treatment-related visceral malformations or variations. Malformations (predominantly associated with the eyes, heart, lungs, and digestive tract) were noted in a total of six fetuses – one control fetus, two fetuses in the 10 mg/kg/day group, and one 25 mg/kg/day fetus. No malformations were observed at 60 mg/kg/day. Part of the median lobe of the liver was dark in two fetuses (1.1%) in a single litter (4.8%). However, the incidences of this variation were considered incidental because they were minor. Furthermore, only a small area (<3 mm) of the liver was affected. The most commonly observed variations included retinal folds, thymic remnant present, and caudate lung lobe absent; however, the incidences of these variations were unrelated to dose. The remaining visceral variations were considered incidental because the fetal and litter incidences were minor and not dose-dependent.

TABLE 7. Visceral findings [% fetuses (litters) affected] [a]				
Observation	**Dose (mg/kg bw/day)**			
	0	10	25	60
No. fetuses (litters) examined	162 (19)	137 (17)	213 (23)	195 (21)
No. heads (litters) examined	176 (19)	65 (17)	99 (23)	92 (21)
Malformations				
Retina, Ocular coloboma (unilateral)	5.3 (5.3)	---	0.3 (4.3) [e]	---
Cleft palate (total)	---	1.2 (5.9) [c]	---	---
Right atrium, enlarged	---	1.2 (5.9) [c]	---	---
Right and left ventricles, enlarged	---	1.2 (5.9) [c]	---	---
Abdomen, fluid filled	---	1.2 (5.9) [c]	---	---
Pulmonary trunk, dilated	---	1.2 (5.9) [c]	---	---
Ascending aorta and aortic arch, dilated	0.8 (5.3) [b]	1.2 (5.9) [c]	0.3 (4.3) [e]	---
Ventricular septum defect in cranial region	0.8 (5.3) [b]	1.2 (5.9) [c]	0.5 (4.3) [f]	---
Lungs lobes, small (all)	---	1.2 (5.9) [c]	0.5 (4.3) [f]	---
Innominate artery, short	---	---	0.5 (4.3) [f]	---
Aortic arch and descending aorta, narrowed	---	---	0.5 (4.3) [f]	---
Thorax, fluid filled	---	---	0.5 (4.3) [f]	---
Colon, enlarged	---	0.5 (5.9) [d]	---	---
Rectum, narrowed	---	0.5 (5.9) [d]	---	---
Left atrium, enlarged	---	---	0.3 (4.3) [e]	---
Left ventricular wall, thin (dorsal part)	---	---	0.3 (4.3) [e]	---
Variations				
Part of median liver lobe, dark (≤3 mm)	---	---	---	1.1 (4.8)
Retina, fold (unilateral/bilateral)	8.1 (21.1)	7.4 (29.4)	7.2 (17.4)	3.5 (14.3)
Thymic remnant present (unilateral/bilateral)	12.5 (63.2)	8.5 (41.2)	11.9 (65.2)	14.6 (66.7)
Caudate lung lobe, absent	4.7 (26.3)	3.2 (17.6)	4.0 (21.7)	2.8 (14.3)

a Data were obtained from Table 10 on pages 71-72 of MRID 47443292.
b, c, d, e, f Findings denoted by the same subscript were observed in the same fetus.
--- No animals affected (i.e., zero incidence)

3. **Skeletal examination:** Incidences of selected skeletal malformations and variations are presented in Table 8. There were no treatment-related skeletal malformations or variations. A single fetus at 60 mg/kg/day had multiple malformations including: 5th and 6th thoracic arches fused; 5th thoracic centrum hemicentric, 6th thoracic centrum bipartite; 1st ribs short (bilateral), two ribs fused (unilateral), and two ribs (5th and 6th) malpositioned. However, the malformations in this fetus were considered incidental because only a single fetus was affected and many of these findings were also noted in historical controls. Incidences of all other skeletal malformations were unrelated to dose. Additionally at 60 mg/kg/day, one fetus had a split (unilateral) frontal, and another had a small interparietal. Although these variations were not reported in the historical control data, they were considered incidental because only a single fetus per finding was affected. Incidences of all other skeletal variations were unrelated to dose and/or were comparable to the incidences observed in the historical control data.

TABLE 8. Skeletal findings [% fetuses (litters) affected] [a]

Observation	Dose (mg/kg bw/day)				Historical controls
	0	10	25	60	
No. fetuses (litters) examined	162 (19)	137 (17)	213 (23)	195 (21)	2015 (225)
Malformations					
Thoracic arches (5th and 6th), fused	---	---	---	0.5 (4.8) [b]	0.0-1.0 (0.0-5.0)
Thoracic centrum (5th), hemicentric	---	---	---	0.5 (4.8) [b]	0.0-1.8 (0.0-16.7)
(6th), bipartite	---	---	---	0.5 (4.8) [b]	0.0-0.4 (0.0-4.2)
Rib (1st), short (bilateral)	---	---	---	0.5 (4.8) [b]	NR
fused, two (unilateral)	---	---	---	0.5 (4.8) [b]	0.0-1.6 (0.0-10.0)
malpositioned, two (5th and 6th)	---	---	---	0.5 (4.8) [b]	NR
Variations					
Frontal, split (unilateral)	---	---	---	1.0 (4.8)	NR
Interparietal, small	---	---	---	0.8 (4.8)	NR
Anterior fontanelle, enlarged	---	---	---	0.7 (4.8)	0.0-3.3 (0-20.0)
Hyoid centrum, incomplete ossification	4.6 (15.8)	2.6 (11.8)	14.1 (34.8)	9.7 (28.6)	0.8-24.3 (4.8-55.0)
unossified	---	---	---	0.7 (4.8)	0.0-4.2 (0.0-25.0)
At least one cervical centrum, incomplete ossification	---	---	0.3 (4.3)	0.5 (4.8)	0.0-0.5 (0.0-5.0) [c]
13th thoracic rib (including bilateral, short unilateral)	63.4 (100)	69.6 (100)	64.0 (95.7)	77.5 (100)	49.6-69.9 (87.0-100)
short (unilateral/bilateral)	16.1 (68.4)	11.2 (52.9)	13.9 (73.9)	8.3 (57.1)	11.5-19.7 (47.6-78.3)
detached(unilateral/bilateral)	11.4 (63.2)	11.7 (52.9)	10.2 (52.2)	12.6 (71.4)	5.1-12.7 (23.8-65.0)
Presence of 27 presacral vertebrae	---	---	2.2 (13.0)	1.4 (14.3)	0.0-2.6 (0.0-12.5)
Presence of 25 presacral vertebrae and only 6 lumbar vertebrae	---	---	---	0.5 (4.8)	0.0-8.2 (0.0-16.7)

a Data were obtained from Table 11 on pages 75-78 and Attachment 4 on pages 260-275 of MRID 47443292.
b Findings denoted by the same superscript were observed in the same fetus.
c Historical control data on page 264 categorize "at least one (except atlas and axis centrum) cervical centrum: incomplete ossification
--- No animals affected (i.e., zero incidence)
NR Not reported

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS= CONCLUSIONS:** It was concluded that the maternal LOAEL was 60 mg/kg/day based on one female aborting, decreased food consumption and body weight gains, and macroscopic changes in the liver (pale liver in 3/23 dams and white foci in 2/23 dams). The maternal NOAEL was 25 mg/kg/day. Fetal development was unaffected by treatment; therefore the developmental LOAEL was not observed, and the NOAEL for developmental toxicity was 60 mg/kg/day, the highest dose tested.

B. **REVIEWER COMMENTS**

1. **Maternal toxicity:** At 60 mg/kg/day, one female (RR4F0461) was killed for humane reasons on GD 27 after having aborted. This animal had decreased food consumption and lost 0.17 kg between GD 24-26. Clinical signs consisted of few feces on GD 26 and 27. Pale liver was noted at necropsy. There were no other treatment-related mortalities.

   At 60 mg/kg/day, 3/23 dams had mucoid feces on two or more occasions from GD 16 onwards, compared to 0 controls. Additionally at this dose, 12/23 females had few feces on

one or more occasions compared to 2/23 controls. Mean maternal body weight gain at 60 mg/kg/day was significantly ($p \leq 0.05$) decreased by 32% for the overall (GD 6-29) treatment period, compared to controls. Corrected (for gravid uterine weight) body weight gain was significantly ($p \leq 0.01$) decreased by 200%. Corresponding to the decreased body weight gains at this dose, food consumption was decreased by 10-30% at each of the intervals throughout treatment, compared to controls. These decreases were significantly different ($p \leq 0.05$) from controls for GD 6-8, 8-10, 22-26, and 26-29. Although liver weights in the treated groups were comparable to controls, pale liver was noted in 3/23 females, and white foci on the liver were found in 2/23 dams at 60 mg/kg/day, compared to 0 controls.

There were no effects on treatment on the 10 or 25 mg/kg/day dams.

The maternal LOAEL is 60 mg/kg/day based on abortion in a single dam, decreased body weight gains, decreased food consumption, and macroscopic findings in the liver (pale liver, white foci). The maternal NOAEL is 25 mg/kg/day.

2. **Developmental toxicity**

a. **Deaths/resorptions:** There were no premature deliveries or complete litter resorptions. There were no effects of treatment on the numbers of litters, live fetuses, dead fetuses, early resorptions, or late resorptions. Additionally, sex ratio and post-implantation losses in the treated groups were comparable to controls.

b. **Altered growth:** There were no treatment-related effects on fetal growth or development as evidenced by fetal body weights in the treated groups that were comparable to controls and the absence of any effects on ossification of the skeleton.

c. **Developmental variations:** There were no treatment-related external, visceral, or skeletal variations.

d. **Malformations:** There were no treatment-related external, visceral, or skeletal malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 60 mg/kg/day.

This study is classified **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3700b; OECD 414) for a developmental toxicity study in rabbits.

C. **STUDY DEFICIENCIES:** No study deficiencies were noted.

Developmental toxicity of Diaminotriazine in rats (MRID 47443292)
Appendix E, pp. 129-132

Control (Group 1)

Dam #	Corpora lutea	Implantations	Early Resorptions	Late Resorptions	Total Resorptions
390	10	10	0	1	1
391	13	13	0		0
392	14	13	0		0
393					0
394	10	10	0		0
395	11	8	0		0
396	9	9	1		1
397	10	10	1		1
398	11	8	1		1
399	13	10	5		5
400	9	6	0		0
401	12	12	3		3
402	12	8	0	1	1
403	8	6	0		0
404	12	12	0		0
405	11	11	0		0
406	8	8	0		0
407	14	12	1		1
409	12	11	0		0
410					0
411					0
412	7	5	0		0
Sum	206	182	12	2	14

10 mg/kg/day (Group 2)

Dam #	Corpora lutea	Implantations	Early Resorptions	Late Resorptions	Total Resorptions
413	13	13	0		0
415	13	13	2		2
416	12	10	0		0
417	11	10	0		0
418	10	9	1		1
419	13	11	0		0
420	14	11	0		0
421					0
422	13	6	1		1
423	8	5	0		0
424	10	9	2		2
425	3	2	0		0
426	11	11	2	1	3
427	13	12	1		1
428	15	11	1		1
429	11	10	0		0
430	9	9	0		0
431					0
432					0
434	11	10	0		0
435					0
Sum	190	162	10	1	11

25 mg/kg/day (Group 3)

Dam #	Corpora lutea	Implantations	Early Resorptions	Late Resorptions	Total Resorptions
436	10	10	0		0
437	12	11	0	1	1
438	15	12	0		0
439	9	9	0		0
440	13	10	1		1
441	11	10	0		0
442	8	8	0		0
443	13	12	0		0
444	12	12	1	1	2
445	11	7	0		0
446	12	11	0	1	1
447	15	13	0		0
448	13	12	0	1	1
449	8	8	0		0
450	9	9	0		0
451	11	10	0		0
452	8	7	0		0
453	14	10	1		1
454	8	7	0		0
455	15	12	2		2
456	11	10	1		1
457	15	11	0		0
458	13	13	0		0
Sum	266	234	6	4	10

60 mg/kg/day (Group 4)

Dam #	Corpora lutea	Implantations	Early Resorptions	Late Resorptions	Total Resorptions
459	13	13			
460	11	11			
462	8	7			
463	12	9			
464	13	11	1		
465	15	12			
466	12	12			
467	9	9			
468	12	8			
469	11	11	1		
470					
471	12	10			
472	13	9			
473	10	10	1		
474	10	10			
475	14	12			
476	10	10			
477	7	7			
478	13	9			
479	11	11			
480	9	9			
481	12	10			
Sum	237	210	3	0	3

Note: Dam #408, 414, 433 were killed in extremis, and Dam #461aborted.
Therefore the data from these animals are excluded